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RESEARCH ARTICLE

First report of *Biscogniauxia mediterranea* (De Not.) Kuntze on *Celtis australis* L. in Bulgaria

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Abstract

This is the first record for Bulgaria of *Celtis australis* as a new host of *Biscogniauxia mediterranea*. Symptoms of the disease (presence of stroma on stems and branches) were found on dried trees in 'Ayazmoto' Park in Stara Zagora in the fall of 2024. The route survey conducted in the spring of 2025 showed that out of a total of 140 trees, 21 were completely dry, and 11 were partially dry. Half of the surveyed trees (70) were determined to be healthy. The detailed survey showed the presence of the black stroma characteristic of the genus *Biscogniauxia* on four dried trees. The results of laboratory analyses give us reason to confirm that the causative agent of European nettle wilt is *B. mediterranea*.

Keywords

Celtis australis, new host, *Biscogniauxia mediterranea*

Introduction

The European nettle tree or Mediterranean hackberry (*Celtis australis* L.) is a deciduous tree with a broad and dense crown that can reach 25-30 m in height. It is widespread in Northwest Africa, Central and Southern Europe and Asia Minor (Delkov, 1984). It grows on hot, rocky slopes, and often on calcareous terrain up to 400-500 m above sea level. A light-loving, heat-loving and drought-tolerant species with valuable wood, undemanding to soil conditions. Outside its natural range, *C.*

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australis is present as an ornamental plant in Central Europe, and is naturalized in Australia and the southwestern United States (Magni, Caudullo, 2016). Due to its undemanding nature, this tree is used for anti-erosion afforestation on difficult terrains. It also has good decorative qualities due to its dome-shaped crown.

No pests or diseases are known of major importance for *C. australis*. However, over the past 50 years, nettle has shown signs of decline, especially in urban areas, due to the effects of climate change and the action of various pathogens (Magni, Caudullo, 2016). Symptoms are evident in drought years and cold winters.

In the database 'Plant parasites of Europe' (Ellis, 2001-2025, consulted April 2025) 43 species of pests, mainly insects, are described for *C. australis*. Among them are 6 fungi, of which 3 species cause leaf spotting (*Sirosporium celtidis* (Biv.) M.B. Ellis), leaf deformation (*Taphrina celtidis* Sadeb.) and powdery mildew (*Erysiphe celtidis* (Schwarzman & Kusnezowa) U. Braun & S. Takam.), 2 species of macromycetes (*Daldinia vernicosa* Ces. & De Not. and *Inonotus rickii* (Pat.) D.A. Reid) and one causative agent of bark necrosis (*Sardiniella urbana* Linald., A. Alves & A.J.L. Phillips).

Biscogniauxia mediterranea (De Not.) Kuntze is a member of the family Xylariaceae (Ascomycota) and is commonly found in the temperate climate of the Mediterranean basin as an endophyte and pathogen of *Quercus suber* L. In addition to *Quercus*, the pathogen can occur on various tree species such as *Acer* spp., *Castanea sativa* Miller, *Fagus sylvatica* L., *Platanus acerifolia* Willd. and *Faxinus excelsior* L. (Patejuk et al., 2022). To this list can be added *Juglans* spp., *Lithocarpus* spp., *Pisonia* spp., *Fagus grandifolia* Ehrh., *Alnus rubra* Bong., *Ribes sanguineum* Pursh, *Ceanothus thyrsiflorus* Eschsch., *Rubus spectabilis* Pursh (Rogers et al., 1999).

For the first time in Bulgaria, *B. mediterranea* was described on *Quercus cerris* L. (Kuthan, Kotlaba, 1988). Recently, the fungus was reported on a new host for the country – *Q. rubra* L. (Georgieva et al., 2024). The disease develops rapidly and causes serious damage and death of trees in the field protective forest belts of Northeastern Bulgaria. In this part of the country, *B. mediterranea* has also been reported on *Q. cerris* (Rosnev, Petkov, 1992), and in Southwestern Bulgaria – on *Q. suber* L. and *Q. pubescens* Willd. (Petkov, Rosnev, 2014; Bencheva, Doychev, 2022).

This article presents *Celtis australis* as a new host for Bulgaria of the fungus *B. mediterranea*.

Material and Methods

Park 'Mitropolit Metodiy Kusev' (better known as 'Ayazmoto') is located in Sarnena Gora (the eastern part of the Sredna Gora mountain). Its altitude varies from 260 to 430 m. The park was established in 1895. Today it has an area of 350 ha, 90% of which is occupied by tree and shrub vegetation, but there are also many areas for walking and recreation. Rare and unique plants for Bulgaria grow in the park, in-

cluding 180 local and exotic tree and shrub species. In 1998, the park was declared a cultural monument of national importance.

In the autumn of 2024, a drying out of a nettle tree (*Celtis australis*) of approximately 60-65 years of age was detected in the 'Ayazmoto'Park (N 42.435924 E 25.622165) in the city of Stara Zagora. Samples of symptomatic damaged tissues (Fig. 1) were taken for analysis in the Forest Phytopathology Laboratory of the University of Forestry in Sofia.

Standard methods were used in laboratory analyses, including the use of humid chambers, isolation on a universal nutrient medium (potato-dextrose agar), description of morphological features and recording the growth rate of the formed mycelium. The growth of mycelial cultures (isolated on PDA after two transfers), at a temperature of 25°C, maintained in a Nahita Drying oven 631 Plus incubator, was monitored daily. The Rayner scale (1970) was used to describe the color of the colonies. Spores of the causative fungus (ascospores released from the stroma and conidia formed on a nutrient medium) were photographed using a KERN & SOHN GmbH ODC 825 camera, compatible with a BOECO BM-180/SP microscope. A minimum of 50 spores from each photograph were measured using the KERN & SOHN Microscope VIS 2.0 Pro program. For a more definitive determination, symptomatic samples of cork oak with proven presence of *B. mediterranea*, collected on 31.05.2024 in an infected stand in the Ograzhden mountain (N 41.417309 E 23.132840), were also analyzed.

Fig. 1. A dried nettle tree in 'Ayazmoto' Park and stromata on its stem

In the spring of 2025, a route and detailed forest pathological examination of nettle trees in linear plantations along the alleys was conducted to determine the extent of drying and the presence of infection. One hundred and forty *Celtis australis* trees were examined in different physiological conditions and at different ages, and the examination began from the building of the Stara Zagora Metropolitanate in the direction of the higher (eastern) part of the park. Trees on both sides of the alley were examined and an assessment of the condition of each tree was made according to the degree of defoliation.

Results

The route survey of the southern nettle plantations in 'Ayazmoto' Park showed that out of a total of 140 trees, 21 were completely dry, and 11 were partially dried. Half of the surveyed trees (70) were determined to be healthy. The overall assessment of the defoliation of nettle trees in the park is 36.25%, which means a deterioration in its condition. The detailed survey showed the presence of black stroma characteristic of the genus *Biscogniauxia* on four dry trees. Peeling bark was noticed on three dry trees, and fruiting bodies of *Schizophyllum commune* on four trees.

The surveyed diameter has varied between 6 and 50 cm. In some places, several trees of the species *Celtis occidentalis* were also noticed, which were not included in the survey, given their good phytosanitary condition. In some places, natural undergrowth of *Celtis australis* is also observed, with the height of young plants reaching about a meter.

Tree dieback is observed mainly on the western side of the alley, in trees with a larger diameter (older). Most trees on the eastern side of the alley are healthy, with more intensive regeneration and many young plants with a small diameter.

When searching for insect pests, no such were found under the bark and in the wood, but only on the leaves - caterpillars of *Libythea celtis* Laicharting (Nymphalidae: Lepidoptera), two of which successfully pupated and imaginated in laboratory conditions.

Microscopic observations of stroma from the collected plant samples proved the presence of the fungus *B. mediterranea*. A large number of perithecia with an irregular cylindrical shape and ostioles raised above the surface were found on the black stroma with a thickness of about 1 mm (0.9-1.1) (Fig. 2). From them, were isolated mycelial cultures.

The data show that the mycelium covers the entire Petri dish in 4-5 days, with the most active growth on the third and the fourth day (Table 1). The average growth rate is 8.7 (8÷10) mm/day. The formed mycelium densely occupies the nutrient medium as part of the hyphae develop on its surface (Fig. 3). The colony has an initially white, and later pink-beige color (61. Rosy buff) with an olive black center (108. Olivaceous Black), olive gray (107. Grey olivaceous) around it and at the periphery of the Petri dish (according to Rayner, 1970). This diversity in the coloration and

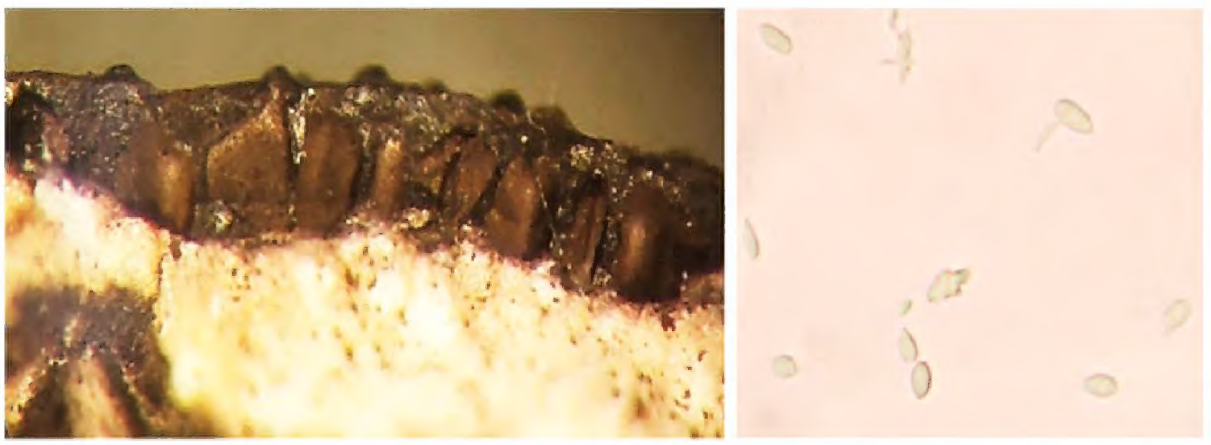

Fig. 2. Transverse section of stroma with perithecia; conidia

Table 1. Mycelial growth (mm/day) in isolates of the fungus placed on 07.4.25

Sample №	1st day	2nd day	3rd day	4th day	5th day	Average growth rate
332 - 1	0	8.3	9.5	19.3	3.0	8.0
332 - 2	0	6.3	6.3	9.5	4.5	8.0
332 - 3	0	9.3	17.5	12.8	-	10.0
Average	0	8.1	11.1	13.8	3.8	8.7

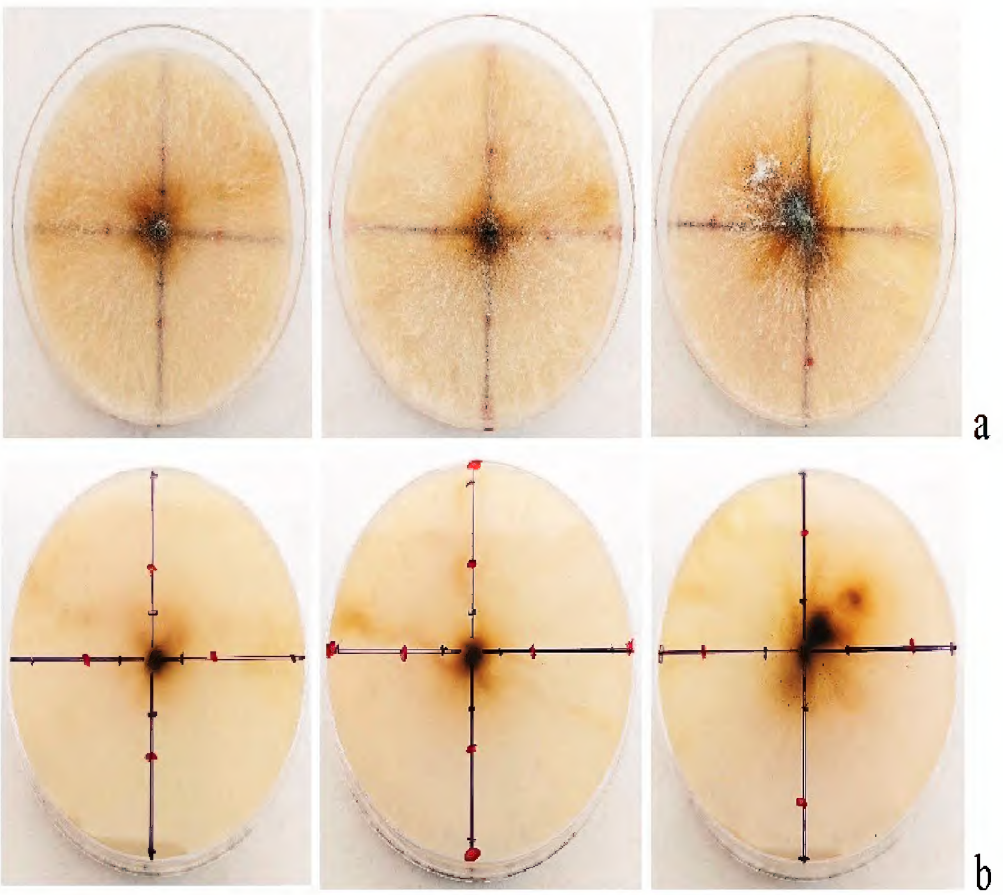

Fig. 3. 10-day-old isolates (a – upper, b – bottom surface)

Table 2. Dimensions (μm) of ascospores and conidia of B. mediterranea in European nettle and cork oak

Host	Spore count	Length (min-max)	Width (min-max)	Spore count	Length (min-max)	Width (min-max)
	ascospores			conidia		
European nettle	207	16.4 (13.0- 20.1)	7.4 (5.8-8.9)	140	6.1 (2.9-9.4)	3.4 (2.6-4.4)
Cork oak	50	16.1 (11.6-20.4)	7.6 (6.0-9.0)	55	6.2 (4.2-9.4)	3.3 (2.7-5.1)

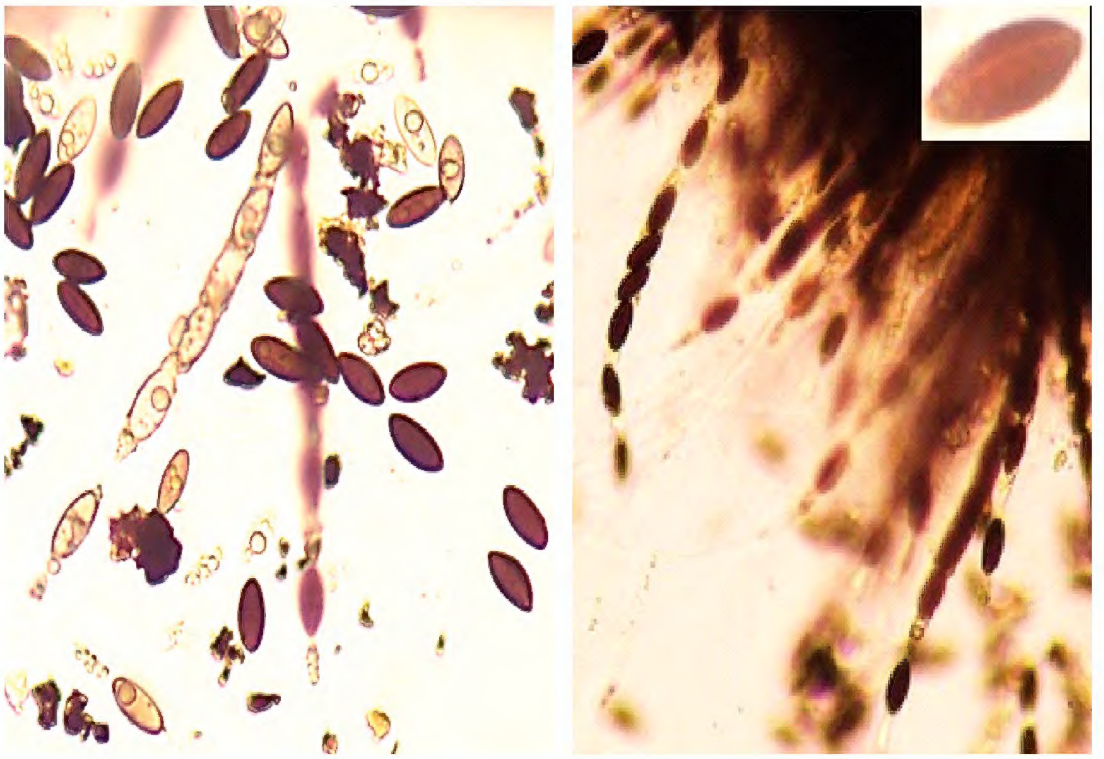

Fig. 4. Asci and ascospores of *B. mediterranea*

structure of the colonies confirms the conclusion of Henriques et al. (2014, 2015) that isolates of *B. mediterranea* show high variability in culture, especially with regard to pigmentation and the presence of aerial mycelium.

Table 2 summarizes the results of measurements of ascospores and conidia. The conidia of the fungus formed on the mycelium in the isolated colonies were unicellular, transparent, elliptical or obovate (Fig. 2), with dimensions of 6.1 (2.9÷9.4) × 3.4 (2.6÷4.4) μm and coincide with those found in the cork oak isolates: 6.2 (4.2÷9.4) × 3.3 (2.7÷5.1) μm. The experiments conducted by Henriques et al. (2015) and analyses of literature data show that the sizes of conidia in *B. mediterranea* varied greatly: (2.51)-5.08-(14.27) × (0.91)-2.07-(3.37) μm. In general, the values obtained by us fall within the ranges indicated by them.

The asci of the fungus (Fig. 4) were cylindrical, with dimensions of 131.9 (99÷157.7) × 9.8 (7.9÷12.7) μm, which were close to those measured by Bencheva,

Doychev (2022) in cork oak from Maleshevska Planina Mt. They contained 8 ascospores, which are dark brown, elliptical, with narrowed ends and a longitudinal slit on one side, as was also found in cork oak in this and previous studies (Bencheva, Doychev, 2022): 122.8 (94.5÷143.1) × 6.8 (5.4÷10.8) μm. Ascospores' dimensions were 16.4 (13÷20.1) × 7.4 (5.8÷8.9) μm and did not differ from those measured in cork oak – 16.1 (11.6÷20.4) × 7.6 (6÷9) μm, respectively. Similar dimensions – 17.6 (13.5÷21.6) × 7.7 (6.8÷9.5) μm – were also established by Bencheva, Doychev (2022).

These results give us reason to confirm that the causative agent of European nettle wilt is *B. mediterranea*.

Discussion

In the global database of the European and Mediterranean Plant Protection Organization (EPPO, 2025), only 7 species of insect pests are described for *Celtis australis*, 5 of which are representatives of the order Coleoptera and 2 species – of the order Hemiptera. In a study of the entomofauna of the European nettle (*Celtis australis*) in Croatia, Slovenia and Hungary (Jurc et al., 2016), seven species of the order Lepidoptera, one from Coleoptera and one from Hemiptera were reported. Defoliators are the most serious pests of *C. australis* also for the conditions of Kenya (Orwa et al., 2009). In its easternmost distribution in Asia Minor, with a possible future expansion into the Mediterranean basin due to hot dry climatic conditions, the nettle is affected by the longhorned beetle *Xylotrechus namanganensis* Heyden (Magni, Caudullo, 2016).

Until the end of the 20th century, few pathogens were reported on *C. australis*. It is known that individual twigs may die from witches' broom, and gall formation on the leaves is much less common than on *C. occidentalis* (Edward et al., 1993).

Climate change, which has intensified in the 21st century, has increased the importance of new phytopathogens. An example of this is the identification by Luongo et al. (2015) of *Phytophthora megasperma* Drech. as the cause of wilting and desiccation of *C. australis* in a nursery in Italy. The decline of nettle is exacerbated by phytoplasmas and the eriophyid mite *Aceria bezzii* Corti, which impair bud development. In southern European cities, the fungus *Inonotus rickii* (Pat.) D.A. Reid has been found to cause rot and necrosis (Magni, Caudullo, 2016). According to literature data, Jurc et al. (2016) found that only a few diseases are known for *C. australis*, such as wood rot of old trees by *Laetiporus sulphureus* (Bull.) Murrill and *Ganoderma applanatum* (Pers.) Pat. The fungus *Helicoceras celtidis* (Biv.) Linder has been isolated from the leaves of *C. australis* in Argentina, and *Ganoderma lucidum* (Curtis) P. Karst. causes rot disease (Orwa et al., 2009). *Perenniporia celtis* Chang et Chou was first described on *C. sinensis* in Taiwan (Chang, Chou, 1999).

Two species of the genus *Biscogniauxia* have been described from species of the genus *Celtis* (Rogers et al., 1999): *Biscogniauxia fuscella* (Rehm) F. San Martín & J.D. Rogers and *Biscogniauxia mediterranea*, which are reported from North America:

B. fuscella – from *Celtis* sp. and *Celtis missisippiensis* (in USA: Texas) and from *Celtis laevigata* (Mexico), and *B. mediterranea* – from *Celtis* sp. (USA: Alabama, 1936, Carver, G. W., as *Nummularia clypeus*). Since *C. australis* is not a native species and has a relatively limited distribution in this continent, it can be assumed that the finds described only to the genus are from other members of the genus *Celtis*.

Rogers et al. (1999) described 49 taxa of the genus *Biscogniauxia*, found on various hosts worldwide. They reported that all members of this genus are weak pathogens, developing on hosts weakened by drought and other factors, and sporulating when the host is in a deteriorated condition or dead. They are adapted to dry or at least seasonally dry habitats.

Georgieva et al. (2024) pointed to recent climate changes towards warmer and drier conditions in the region of Northeastern Bulgaria and the subsequent increase in water stress on trees as the main reason for the spread of *B. mediterranea* on *Quercus rubra* in the field protective forest belts and forest plantations. Given the increasing number of reported new hosts of the fungus, it can be said that these changes are the leading reason for the increase in aggressiveness of this phytopathogen in *C. australis* that we have found.

B. mediterranea is a species with increasing economic impact due to climate change and according to Patejuk et al. (2022), who conclude that the danger of its range expansion could be significant, given rising annual temperatures and regular droughts.

Conclusion

Although no dangerous pathogens have been described for *Celtis australis*, a considerable number of insect pests, mainly defoliators, have been identified on it. They do not kill the attacked tree, but disrupt its normal physiological state and exacerbate the stress caused by adverse climate changes. These factors predispose the transition of some endophytically developing microorganisms from a latent to a parasitic state and their transformation into a threat to the survival of their hosts. Such is the fungus *B. mediterranea*, identified by us on *C. australis* on dead trees in 'Ayazmoto' Park in Stara Zagora.

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